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Breinlia tinjili sp. n. (Filarioidea: Onchocercidae), from the Malaysian Field Rat, *Rattus tiomanicus*, on Tinjil Island, West Java, Indonesia

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ABSTRACT: *Breinlia tinjili* sp. n. (Filarioidea: Onchocercidae) is described from the thoracic and abdominal cavities of 4 rats, *Rattus tiomanicus* Miller, 1900, from Tinjil, an uninhabited island off the west coast of Java, Indonesia. *Breinlia tinjili* adult worms have 12 to 13 asymmetrical perianal papillae, a wide cup-shaped capitulum of both spicules, a 2.5:1 spicule ratio, a thick trilobed gubernaculum, and numerous cuticular bosses scattered along the dorsal and ventral surface of the worm. Microfilaria are unsheathed, measure between 273 and 300 μm in length, and have 6–9 tail nuclei in a single row and a long lashlike tail. Descriptions are most similar to *B. spratti* and *B. booliati*, but differ in the following characteristics: the adults are smaller, the gubernaculum is trilobed, paired spicules are smaller, males have an extra pair of postanal papillae, and the microfilariae are much larger in *B. tinjili* than in *B. booliati*. *Breinlia spratti* has a reduced number and different arrangement of male perianal papillae, a smaller nongrooved gubernaculum, and smaller spicules compared to *B. tinjili*. This new species appears to share a number of close characters with other *Breinlia* species previously described from rodents. The diagnostic value of these characters is discussed.

KEY WORDS: Onchocercidae, *Breinlia tinjili* sp. n., *Rattus tiomanicus*, Indonesia, morphology.

During a biomedical expedition to Tinjil Island on 20–24 March 1989, U.S. Naval Medical Research Unit No. 2 staff, conducting routine capture and processing of native rodents, found adult filariid worms in the thoracic and abdominal cavities of 4 Malaysian field rats, *Rattus tiomanicus* Miller. Subsequent examinations of these specimens revealed that they represented a new species within the genus *Breinlia* which is described herein. Tinjil (6°58′S, 105°45′E) is an uninhabited coral island of approximately 600 hectares of undisturbed tropical forest located in the Indian Ocean 10 km off the south coast of West Java, Republic of Indonesia.

Materials and Methods

Adult worms were removed from the thoracic and abdominal cavities of euthanized rats, fixed in hot 10% formalin and preserved in 70% ethanol/5% glycerin. All specimens were examined using a temporary lactophenol wet-mount technique (Partono et al., 1977). Microfilariae were obtained from a thick blood smear and stained for 15 min with Giemsa diluted 1:15 with pH 7.2 buffer. Drawings (Figs. 1–6) were made with the aid of a camera lucida. All measurements are expressed as means (range) and are given as length by width in micrometers (μm) unless otherwise indicated.

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Results

Breinlia tinjili sp. n. (Figs. 1-6)

HOST: Malaysian field rat, *Rattus tiomanicus* Miller, 1900.

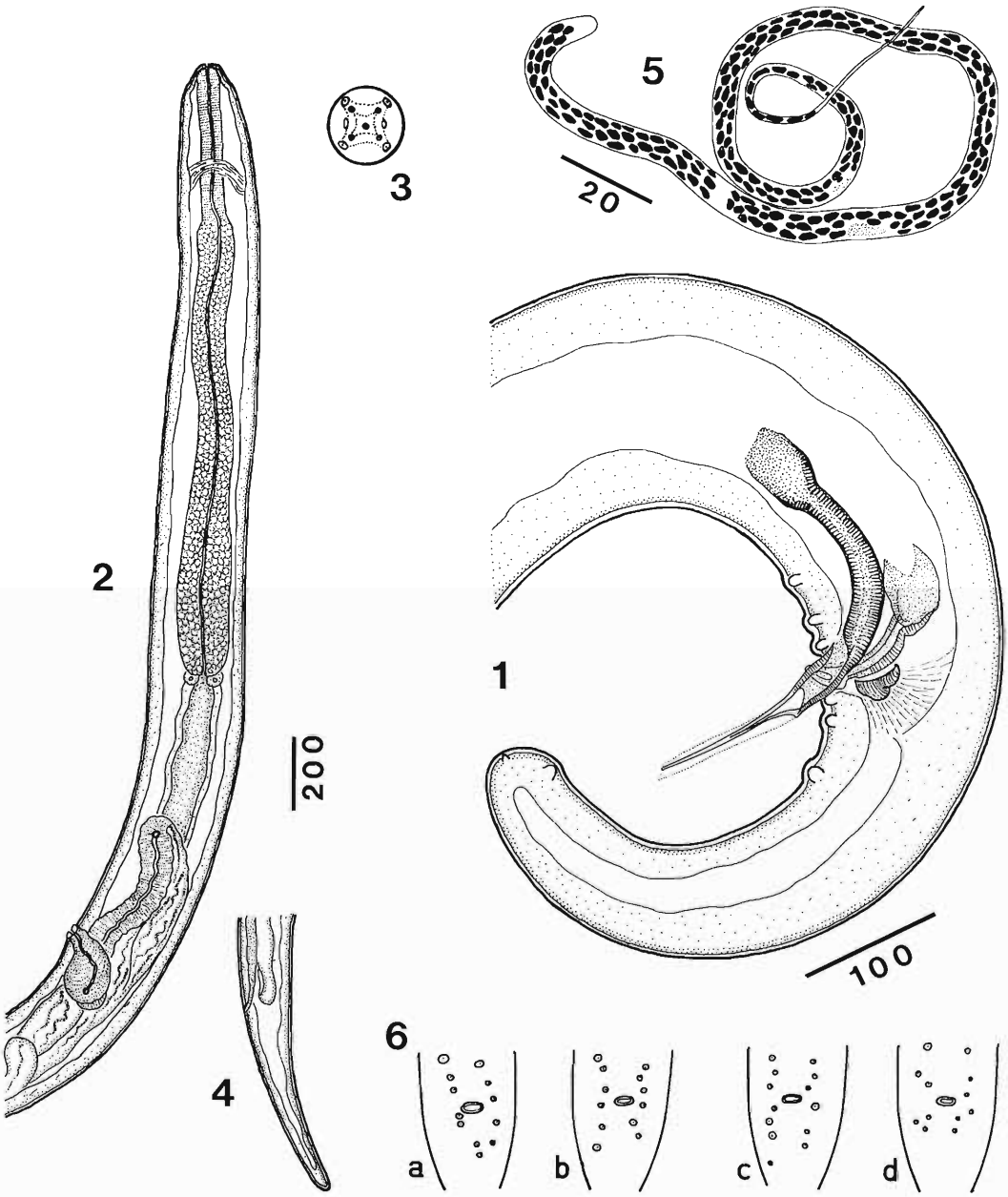
LOCATION: Thoracic and abdominal cavities.

LOCALITY: Tinjil Island, SW Java, Indonesia (6°58′S, 105°45′E).

SPECIMENS DEPOSITED: USNM Helm. Coll. nos. 80943 for both holotype male and allotype female, and 81169 for male and female paratypes, all in 70% ethanol/5% glycerin, and 4 slides of microfilariae (syntypes), Giemsa-stained, are deposited in the U.S. National Parasite Collection, Beltsville, Maryland, 20705 U.S.A.

DESCRIPTION: Adults filiform, yellow to white when fixed. Anterior and posterior ends blunt. Mouth simple, without lips. Buccal cavity indistinct with cuticular ring at base. Head with 4 pair submedian cephalic papillae arranged in 2 rings of 4 with 2 lateral amphids (Fig. 3). Cuticle finely striated transversely with small, slightly curved cuticular bosses covering worm at midbody to near cloacal aperture. Esophagus with unequal (ratios) anterior muscular and posterior glandular portions, the latter being slightly wider. Nerve ring at middle of muscular region of esophagus. Vulva postesophageal.

MALE (based on 5 mature specimens): Body 37.8 (31.1–42.2) mm by 150 (130–170) at level of nerve ring; width increasing posteriad, 157



Figures 1–6. ***Breinlia tinjili*** **sp. n. adults and microfilaria from a rat from Tinjil Island, West Java. All scale bars in μm. 1. Caudal end of male, lateral view, showing left and right spicules, gubernaculum, and arrangement of pre- and postanal papillae. 2. Anterior region of female, lateral view, showing esophagus and ovejector. 3. En face view of female showing arrangement of 4 pairs submedian cephalic papillae and 2 lateral amphids. 4. Caudal end of female, lateral view, showing position of anus. 5. Unsheathed microfilaria. 6. a–d. Caudal end of male, ventral view, showing various arrangements of perianal papillae.**

(140–180) at level of glandular region of esophagus, 170 (150–200) at esophageal-intestinal junction and 202 (185–215) at level of midworm, gradually decreasing to 105 (90–110) at level of cloaca. Esophagus 1,651 (1,500–1,825), anterior muscular portion 510 (360–750) by 46 (35–55) (30% of entire length) and posterior glandular portion 1,141 (1,050–1,235) by 94 (90–100).

Nerve ring 264 (225–325) from cephalic end. Tail coiled (1–2 turns) into tight spiral 363 (310–430). Asymmetrical perianal papillae present: 6 preanal, 6 to 7 postanal, and 1 pair each at terminal and subterminal region of tail. Ratio of width at cloaca to length of tail 1:3.5 (2.8–4.1). Left and right spicules (Fig. 1) unequal in length and dissimilar in appearance. Left spicule 316 (295–340), composed of 4 sections; a thick-walled, tubular, striated, and granular proximal portion 159 (150–175); open, expanded, and cup-shaped capitulum 46 (40–50) wide; a thin-walled, semicircular, hyaline midsection 60 (50–70), and a narrow rodlike distal portion 97 (75–120) ending bluntly. Sheath of left spicule evident when extruded. Right spicule 130 (124–135) curved ventrad, divided into 3 distinct portions; a proximal section 56 (50–65), enlarged and rounded with fine striations on wall; a middle cylindrical thick-walled section 48 (35–53), and a distal cylindrical thick-walled section 27 (20–50) ending in a spatulate tip. Left to right spicule ratio 2.5 (2.2–2.6):1. Gubernaculum heavily sclerotized and trilobed in lateral view 36 (33–40) by 17 (15–20).

FEMALE (based on 11 gravid specimens): Body 77.6 (53.6–101.4) mm by 187 (160–210) at level of nerve ring; width increasing posteriad, 223 (170–260) at level of glandular region of esophagus, 246 (210–290) at esophagointestinal junction, 267 (230–300) at vulval opening, 338 (300–400) at midbody, gradually decreasing to 152 (100–230) at posterior uterine coils and 116 (100–140) at anal opening. Esophagus (Fig. 2) 1,819 (1,520–2,005), anterior muscular region 459 (400–675) by 58 (50–80) (25% of entire length) and a posterior glandular region 1,360 (1,120–1,570) by 132 (100–150). Nerve ring 261 (230–300) from cephalic end. Vulva opening as a transverse slit immediately posterior to esophageal-intestinal junction, 2,828 (1,930–3,680) from cephalic end. Ovejector pear-shaped, 148 (100–200) by 103 (75–125), with muscular wall. Vagina directed anteriorly, flexing and looping posteriad before receiving uteri at varying distances (500–1,000) below vulva. Uteri paired, loosely entwined, joining oviduct and extending anteriorly to within 2,287 (1,500–2,600) from cephalic end; posterior coil extending to within 990 (520–1,250) from tip of tail. Tail 638 (510–750) with blunt end, and 2 pairs of very small phasmids located subterminally. Viviparous.

MICROFILARIA (based on 30 specimens): Body slender, unsheathed, 285 (273–300) long. Width at level of first nucleus 5.0 (5.0), nerve ring 5.1 (5.0–6.0), excretory pore 5.3 (5.0–6.0), and anal pore 4.1 (4.0–5.0). Cephalic space length 5.4 (3.0–5.0). Distance from anterior end to nerve ring 52 (50–55), excretory pore 78 (73–90), anal pore 203 (194–215), and last nucleus 251 (238–265). Tail 33 (30–37) from last nucleus to posterior tip, tail nuclei, 6–9 in single row (Fig. 5).

Discussion

Breinlia tinjili shares a number of close morphological characters with other species in the genus described from rodents, specifically, *B. booliati* Singh and Ho, 1973 and *B. spratti* Bain et al., 1979. Differentiation can be made based on 1 or more of the following characteristics: the shape, length, and width of the gubernaculum and the length of microfilaria and adults. Additionally, *B. tinjili* differs from *B. booliati* and *B. spratti* in that adult males have an extra pair of postanal papillae with a complete papillar arrangement of 6 asymmetrical preanal, 6–7 asymmetrical postanal and 1 pair each of terminal and subterminal papillae (Fig. 6a–d). A single pair of adanal papillae as described for *B. booliati* was observed in 1 male specimen. Papillae arrangement appears variable and degrees of symmetry make it difficult to accurately judge positions of papillae respective to the anus. *Breinlia spratti* has the majority of papillae aligned along the midline with the anus.

The spicule ratio of *B. tinjili* is 2.5:1 and is similar to that of *Breinlia manningi* Bain et al., 1981, *B. spratti,* and *B. booliati.* However, the average lengths of both spicules were found to be intermediate between the 3 species. The capitula of both spicules are more expanded than illustrated for the other 3 species. The gubernaculum is wide, grooved and trilobed with an average length of 36 μm. *Breinlia spratti* has a smaller structure (24 μm) that is smooth in appearance (lacks a ventral groove). *Breinlia booliati* is of similar length but is distinctly bilobate. Adult worms are similar in length to those of *B. spratti,* whereas adult *B. booliati* are considerably larger.

The cuticle has fine, transverse striations and small, elongate refractile bosses (longitudinal crests) beginning along the midregion of the body and terminating just anterior to the cloacal aperture. Cuticular bosses show irregular spacing and positions, being scattered on ventral and dorsal surfaces and reaching the lateral edges. This cuticular ornamentation is similar to that

Table 1. Selected character comparisons of 3 *Breinlia* species.

	B. tinjili[1]	*B. booliati*[2]	*B. spratti*[3]
Length (mm)			
Female	78 (54–101)*	197 (168–213)*	74
Male	38 (31–42)*	64 (46–77)*	31
Maximum width (μm)			
Female	338 (300–400)*	480 (370–521)*	330
Male	202 (185–215)*	245 (197–297)*	235
Length (μm)			
Left spicule	316 (295–340)*	371 (349–385)*	292
Right spicule	130 (124–135)*	144 (118–167)*	110
Gubernaculum (μm)			
Length	36 (33–40)*	35 (30–39)*	24
Width	17 (15–20)*	9 (6–12)*	—
Shape	3-lobed	2-lobed	smooth
Papillar arrangement			
Preanal	6	6	6
Adanal	—	2	—
Postanal	6–7	4	6
Tail tip	2 pair	2 pair	2 pair
Microfilariae (μm)			
Length	273–300	188–206	270–320
Width	5.3 (5–6)*	3.9 (3–5)	5.5

[1] Purnomo and Bangs sp. n. (1996).
[2] Singh and Ho (1973).
[3] Bain, Tibayrenc, and Mak (1979) (range measurements not given).
* Mean (range).

described for *B. spratti* and *B. booliati* with the notable exception that bosses are far less dense on the caudal extremity (area rugosa) of both the male and the female. The long and slender microfilaria of *B. tinjili* (285 μm [273–300]) is longer than other known species, excluding *B. spratti* (270–320) and *B. dendrolagi* Solomon, 1933 (280–300). The combination of aforementioned morphologic characters allows for separation of *B. tinjili* from the other closely related *Breinlia* (Table 1).

Based on the study of marsupial filariid species from the Australasian region, Spratt and Varughese (1975) believed there was no morphological or biological justification for maintaining the genus *Breinlia,* Yorke and Maplestone, 1926 and subsequently placed the genus as a synonym of *Dipetalonema.* However, Bain et al. (1979) have retained the genus and its previous members as followed in this discussion. Of the 13 previously reported species of *Breinlia* (Bain et al., 1981), only *B. booliati* has been recovered from both *Rattus* and *Callosciurus* (Singh and Ho, 1973; Mak and Lim, 1974; Lim et al., 1975, 1978; Bain et al., 1979). Close morphological similarities are seen between *B. tinjili, B. spratti,* and *B. manningi,* the last 2 parasites found in rodents in the family Sciuridae. The finding of *B. tinjili* in Muridae from Tinjil and nearby Deli Island represents the second report of a *Breinlia* filariid found in Indonesian mammals and the second species report of this genus from *Rattus* spp. Lim et al. (1978) reported finding *B. booliati* in *R. tiomanicus* from Ciloto, central-west Java, Indonesia. Although presumably true, only 1 complete male and 1 complete female were available for examination, and perianal papillae, cuticular ornamentation, and the microfilaria were not described.

Biological characteristics vary as well. The microfilarial periodicity of *B. tinjili* in the natural host *R. tiomanicus* is aperiodic. This is in contrast to the nocturnal periodicity described in *B. booliati* and its natural host, *Rattus sabanus* (Thos.) (Yap et al., 1975). Unlike *B. booliati,* which can develop in the albino rat, we were unsuccessful at establishing infection by subinoculation of *B. tinjili* in laboratory rats (Atmoseodjono and Purnomo, unpubl.). A potential natural vector for this filariid appears to be *Aedes*

(*Stegomyia*) *albolineatus* (Theobald) which is active in high density during daylight hours most of the year on both islands. Normal development of microfilariae to infective-stage larvae has been observed in the fat body cells of *Ae. albolineatus* and experimental laboratory mosquitoes, *Ae.* (*Finlaya*) *togoi.* Full development to the infective stage takes about 10 days, similar to descriptions by Ho et al. (1973).

The diagnosis of new species within the genus *Breinlia* can be a difficult task because many characters, such as papillar arrangement and spicular size and appearance, can be variable. Because of the close morphological similarity between *B. tinjili* and other *Breinlia,* especially *B. spratti,* found naturally in rodent hosts, a close ancestral relationship can be hypothesized. Based on the known geographic distribution of these filaria, we surmise a relatively recent allopatric speciation has taken place. It would be of interest to look at various molecular levels of expression in this group to help estimate genetic distances between species or investigate questions of possible conspecificity.

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Literature Cited

Bain, O., M. Tibayrenc, and J. W. Mak. 1979. Deux especes de *Breinlia* (Filarioidea) chez un Ecureuil en Malaisie. Bulletin Museum national d' Histoire naturelle, Paris, 4e ser., 1, 1979, section A, 1:191–197.

———, G. Petit, N. Ratanaworabhan, S. Yenbutra, and A. G. Chabaud. 1981. Une nouveile filaire d' ecureuil en Thailande, *Breinlia* (B.) *manningi* n.sp. et son développement chez *Aedes* (1). Annales de Parasitologie Humaine et Comparée (Paris) 56: 193–201.

Ho, B.-C., M. Singh, and B.-L. Lim. 1973. Observations on the development of a new filariid (*Breinlia booliati,* Singh and Ho, 1973) from a rat *Rattus sabanus* in the mosquito *Aedes togoi.* Journal of Helminthology 47:135–140.

Lim, B. L., J. W. Mak, and S. Nalim. 1978. New hosts of *Breinlia booliati* with observations on its distribution in Southeast Asia. Southeast Asian Journal of Tropical Medicine and Public Health 9:526–528.

———, ———, Mulkit Singh, B.-C. Ho, and E. H. Yap. 1975. Distribution and ecological consideration of *Breinlia booliati* infecting wild rodents in Malaysia. Southeast Asian Journal of Tropical Medicine and Public Health 6:241–246.

Mak, J. W., and B.-L. Lim. 1974. New hosts of *Breinlia booliati* in wild rats from Sarawak with further observations on its morphology. Southeast Asian Journal of Tropical Medicine and Public Health 5:22–28.

Partono, F., Purnomo, D. T. Dennis, S. Atmosoedjono, S. Oemijati, and J. H. Cross. 1977. *Brugia timori* sp. n. (Nematoda: Filarioidea) from Flores Island, Indonesia. Journal of Parasitology 63:540–546.

Singh, M., and B.-C. Ho. 1973. *Breinlia booliati* sp. n. (Filarioidea: Onchocercidae) a filaria of the Malayan forest rat, *Rattus sabanus* (Thos.). Journal of Helminthology 47:127–133.

Spratt, D. M., and G. Varughese. 1975. A taxonomic revision of filarioid nematodes from Australasian marsupials. Australian Journal of Zoology, Supplementary Series 35:1–99.

Yap, E.-H., B.-C. Ho, M. Singh, K.-L. Kang, and B.-L. Lim. 1975. Studies on the Malayan forest rat filaria, *Breinlia booliati:* periodicity and microfilaraemic patterns during course of infection. Journal of Helminthology 49:263–269.